Dioxin Toxicity: New Studies Prompt Debate, Regulatory Action

New data on dioxin's effect on humans, a clearer picture of the cellular events it precipitates, and new animal toxicity studies may provide EPA with a firmer basis for regulation

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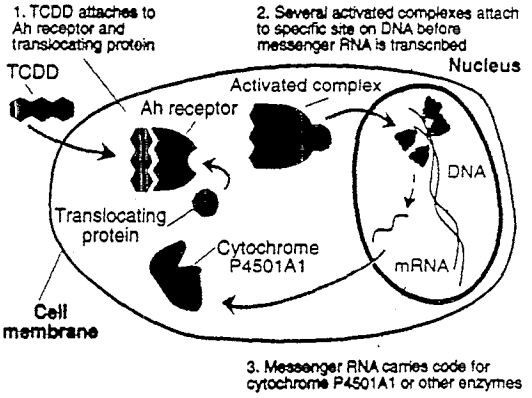
Of all the chemicals that have been tossed into the caldron of public anxiety, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD, has achieved the most notoriety and evoked the greatest fears for the longest period of time. Beginning with herbicide use during the Vietnam War, propelled by Love Canal and Times Beach, and sustained by reports of contamination from city incinerators and paper mills, TCDD essentially defines chemical contamination for most people.

TCDD was first implicated as the culprit responsible for illnesses among chemical industry workers who produced 2,4,5-trichlorophenol. However, the chemical was thought to be only of industrial concern until the mid-1970s. In 1976, an explosion at a chemical plant spread from 1 to 4 lb of TCDD over the residents of the town of Seveso, Italy. High TCDD levels were recorded in people from the area. Shortly afterward, many Vietnam veterans began wondering if their range of illnesses could have been caused by exposure to the herbicide agent orange which was known to be contaminated with TCDD.

When TCDD was found to be part of the highly reported and emotionally charged hazardous-waste leakage that resulted in evacuation of the community of Love Canal, Niagara Falls, N.Y., in 1980, and evacuation and purchase by the federal government of the entire town of Times Beach, Mo., in 1983, it became firmly impressed in people's minds that here was a chemical problem of elephantine proportions. But just as the four blind men each described an elephant differently because they were each touching different parts of the animal, so different scientists, government officials, corporations, and environmental activist groups describe the hazards of TCDD differently. It is only recently that a clearer picture of this creature has emerged, and while it may not be the mammoth once believed, it is no mouse either.

Several events have come together over past months that have helped focus the picture. One is a symposium held at the Banbury Center at Cold Spring Harbor Laboratory, New York, in October that took a critical look at the state of the molecular science of TCDD. The conclusions reached there are extending far beyond research laboratories. Another is the release of significant epidemiological research data involving people exposed to TCDD aimed at determining whether or not they are suffering illnesses as a result. The Environmental Protection Agency, some say in response to these events, has

Receptor-mediated TCDD action requires several steps



In the cell, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) binds strongly to a soluble intracellular protein designated the Ah (for aryl hydrocarbon) receptor, which then binds to a translocating protein that carries the "activated complex" into the cell nucleus. Several activated complexes bind to specific DNA sequences, distorting the DNA chain. Ensuing events lead to transcription of messenger RNA that codes for cytochrome P4501A1 or other enzymes

undertaken an important review of TCDD toxicity that may lead it to change its policies on carcinogenicity.

Much of TCDD's dreadful image stems from its label as "the most toxic synthetic chemical known to man." It is widely conceded that this label is attributable to early research that found that as little as 0.6 μg per kg body weight could kill 50% of guinea pigs exposed to TCDD. Research by Dow Chemical scientists in 1978 found that the chemical was also a potent carcinogen in rats. These facts, repeated in virtually all newspaper and television reports about TCDD, made the chemical the most feared of all contaminants.

Subsequent decisions to lower TCDD concentration in the environment led to the banning of products such as 2,4,5-trichlorophenoxyacetic acid, a herbicide contaminated with TCDD. Production of other products, especially those that used 2,4,5-trichlorophenol as a precursor, was stopped. When it was discovered that municipal and hazardous-waste incinerators were large sources of TCDD in the environment, new controls were implemented to limit TCDD emissions. Use of leaded gasolines was also found to be a prime source of environmental TCDD, and EPA's phaseout of leaded gasoline has also resulted in reduced emissions.

Based on its current risk assessment model, EPA has calculated that the tolerable daily intake of TCDD for humans is 0.006 picograms per kg body weight per day. This level would supposedly result in a one in 1 million chance of excess cancer from TCDD. But this level is far, far below the 1 to 3 picograms per kg body weight per day that people are estimated to be actually ingesting. Other nations, using different risk assessment models, have calculated the tolerable intake level much higher. Germany uses 1 picogram, the Netherlands 4, and Canada and the World Health Organization use 10 picograms per kg body weight per day.

Because health problems associated with TCDD exposure first came to light as the result of chemical industry accidents in the 1950s, the industry has always been a target of blame for environmental contamination. Dow Chemical, Monsanto, and six other chemical companies that made agent orange for the federal government during the Vietnam War were the defendants in a major lawsuit that was settled when the companies agreed to pay $180 million to veterans claiming illnesses resulting from exposure to the herbicide. TCDD has subsequently been at the root of thousands of injury lawsuits, including cases filed by people who lived at Times Beach and Love Canal.

A significant decision was handed down this summer when the Illinois Appellate Court of the 5th District overturned a $16.25 million punitive damage verdict against Monsanto (C&EN, June 24, page 6). The jurors had found no injuries among people claiming to have been injured by dioxin exposure following a train accident, but decided to punish Monsanto anyway. The appeals court judges ruled that if there are no injuries, punitive damages cannot be exacted. A request to have the appeal reheard was also rejected.

Some attorneys specializing in personal injury cases believe this decision may be a turning point for TCDD and other chemical injury cases. David G. Owen, law professor at the University of South Carolina, for instance, says: "The decision may stand as a declaration of judicial intolerance to the use of courtrooms as arenas for subjecting U.S. industry to political and ideological persecution."

David Snively, litigation counsel for Monsanto, has already seen an actual drop-off in dioxin litigation. The company ceased making chlorinated phenols a decade ago, and Snively says about the only relationship Monsanto now has with TCDD is some research. "There has been a fall-off in litigation involving Monsanto, and we have no active lawsuits," he says. He has noticed that new lawsuits seem to be cropping up in the paper and pulp industry.

The paper industry was surprised in 1985, when TCDD was discovered in the effluent and sludge from paper mills. It was found that chlorine bleaching of the pulp caused TCDD formation. Claims soon arose that TCDD was leaching into children's milk cartons, and EPA found itself faced with a lawsuit brought by the National Wildlife Federation and the Environmental Defense Fund demanding regulation of TCDD in the mill effluent.

Although concerns about human health hazards from paper products have subsided, the environmental concerns have spurred paper manufacturers to treat effluents to reduce TCDD and to look into process changes to decrease TCDD production. The industry uses about 14% of all chlorine produced in the U.S. and concentrations of TCDD in the effluents are in the parts-per-quadrillion range. The Chlorine Institute, which represents the chlor-alkali industry, is very worried about this problem, but maintains that the parts per trillion and lower concentrations of dioxins and furans generated by papermaking do not pose a health or environmental threat.

However, the Chlorine Institute was one of the sponsors of the Banbury Center meeting. The conference, which brought together 38 invited scientists from the U.S. and Europe, was actually the second meeting held to discuss TCDD; the first was in 1985. In addition to the Chlorine Institute, the meeting was cosponsored by EPA and FDA. All scientific aspects of the issue were laid out at the conference, and by the end, there was some general agreement on several issues.

Michael A. Gallo, associate professor of research at the Robert Wood Johnson Medical School in New Jersey, was one of the conference organizers. "We met for four days

TCDD toxicity varies greatly among species

Species	LD_{50} (μg per kg)
Guinea pig	0.6–2.5
Mink	4
Rat	22–320
Monkey	<70
Rabbit	115–275
Mouse	114–280
Dog	>100–<3000
Hamster	1150–5000

Source: Environmental Protection Agency

and we reviewed all the science, and we reviewed a lot of the mathematical and biological models that went into it. We came away with the idea that the primary events in and around dioxin were related to this thing we call the Ah receptor." Gallo says the meeting did clarify the scientific issues in that "it helped focus the thought patterns of where a lot of people want to go over the next year, and I think it focused where [EPA Administrator] Bill Reilly and EPA have to put their energies."

Houk: improve risk assessments

Overshadowing everybody's concern over TCDD is that, despite the compound's obvious toxicity to animals, no clear-cut human health problems were associated with TCDD exposure, even though a number of human exposure incidences were known. Epidemiologic studies undertaken to identify any major health problems always had so many shortcomings that researchers could not say whether TCDD had an effect or not. This year two studies have brought the answer closer.

The Air Force Ranch Hand study, begun in 1978, is the oldest study of people heavily exposed to TCDD and the most controversial. An ongoing study, it continues to draw severe criticism. Study participants are members of Operation Ranch Hand, the group that actually handled and sprayed agent orange over the fields and forests in Vietnam from 1962 to 1971. Agent orange was used to defoliate forests and kill some crops in Vietnam for the protection of U.S. forces. It was a 50-50 combination of the herbicides 2,4,5-T and 2,4-D and was contaminated with about 2 ppm or less of TCDD. The original study involved 1242 Rand Hand personnel, although the actual number of participants varies somewhat from examination to examination.

The strongest critic of the Ranch Hand studies has been the American Legion, which represents U.S. veterans who served in Vietnam. The American Legion says the Ranch Hand studies are not designed well enough to see excess diseases because of too few participants and that the classification for exposure was improperly done. It further charges that the Air Force has actually suppressed adverse health effects findings. Despite the Air Force's claims of no significant illness such as cancer or reproductive effects, the legion, with backing from some researchers it has supported to do its own reviews, believes the Ranch Hand personnel have suffered from TCDD exposure.

No one has doubted that those in the Ranch Hand study were exposed to TCDD. In the Air Force's latest report, issued in March, the blood levels of TCDD were measured in 866 Ranch Hand veterans and 804 comparison veterans for the first time, and those levels were correlated to any health effect they could find. Values for TCDD in blood serum ranged from 0 to 618 ppt in Ranch Hand personnel, but the median value was 12.8. Median value of the comparison group was 4.2. Aside from some other, very minor correlations, the report found a significant increase in body fat and diabetes that correlated with TCDD concentration.

This was a surprise, according to William H. Wolfe, chief of the epidemiology research division of Armstrong Laboratory at Brooks Air Force Base in Texas, which is the lab responsible for Operation Ranch Hand. "This obviously needs a closer look," Wolfe says. "We are doing another examination of the same group starting early next year. We are changing and extending our physical examination tests to look critically at the diabetes and to get more answers." Wolfe adds that this finding, while serious, did not get much attention perhaps because "it is not one of the diseases that people had a vested interest in." The American Legion criticisms of the March Ranch Hand report did not even mention the diabetes finding.

Wolfe admits that some of the criticisms of the Ranch Hand study are valid. The small sample size is a problem for identifying increases in diseases like rare cancers. "These limitations were all laid out at the beginning," Wolfe says; "we just got all the Ranch Handers there were." Statistically, he says, "the study has only a 50% ability to detect a doubling of rare cancers like soft tissue sarcoma. And that's too low." For detecting a rise in all cancers, the odds are better. "If we take all cancers together, we have a 90% chance of detecting a 50% increase," he says.

The complaints that data have been deliberately withheld are groundless, Wolf says. He points out that all the data go through a review committee that makes recommendations for additions or changes, which are taken care of before the report goes to the Surgeon General. Given the advisory committee oversight, Wolfe does not see how data could be left out, especially since Sen. Tom Daschle (D.-S.D.), concerned that the Ranch Hand study might be getting inappropriate advice, changed the board composition so that 30% of its members were nominated by veterans groups.

One of the most emotional issues of TCDD has followed in the wake of the Ranch Hand study. That is, whether other soldiers who served in Vietnam suffered from exposure to agent orange and the dioxin that contaminated it. Study after study by the government have shown that men and women serving in Vietnam were not exposed to high levels of TCDD. The Centers for Disease Control tried more than once to find a correlation between Vietnam service and health problems or blood serum dioxin concentrations and could not find one. The Department of Veterans Affairs also has stud-

ied veterans and concludes that agent orange caused no health problems.

The most recent report came from Han K. Kang, head of the Office of Environmental Epidemiology at DVA. Published in March, Kang's study measured blood serum TCDD levels in 36 veterans who, from as best as could be determined from records of agent orange spraying and troop positions, might have been exposed to the herbicide. "We tried to come up with an exposure assessment based on this," Kang says. "We couldn't find any increased exposure. The difference between their body burdens of TCDD was not significant" compared with the control group. Kang says DVA is finished with efforts to determine TCDD levels in Vietnam veterans. "We have done all we can in dealing with the veterans," he says. "We are satisfied with the conclusion that there is no significant elevation in TCDD levels."

TCDD has several toxic effects

- Death
- Wasting syndrome
- Thymic atrophy
- Splenic atrophy
- Testicular atrophy
- Liver enlargement, fatty deposits, necrosis
- Hyperplasia: gastric mucosa, urinary tract, bile duct
- Squamous metaplasia: meibomian glands, ceruminous glands
- Chloracne: hyperplasia, hyperkeratosis, altered pigmentation
- Teratogenesis
- Carcinogenesis
- Immunosuppression
- Enzyme induction
- Biochemical effects

Source: Environmental Protection Agency

One striking finding by Kang is that the body burden of TCDD in the population appears to be falling. The samples he measured were collected in the early 1970s by the National Human Adipose Tissue Monitoring Program. Kang reported mean background levels of about 12 ppt for these samples. More recent work, such as that done by CDC, found background levels of TCDD at 5 to 7 ppt. "We are speculating that the environmental TCDD levels are going down, for whatever reason. Other countries, like Sweden, have also reported that organic compounds, including TCDD, are declining," Kang says.

Another reason DVA does not feel it needs to do more studies is a change in administrative policy made in 1990 by Secretary Edward J. Derwinski. In response to a lawsuit filed in California, the department has begun offering compensation to Vietnam veterans who suffer from diseases thought to be caused by agent orange exposure, even though no such link has ever been proven. The diseases currently accepted are soft tissue sarcoma, non-Hodgkins lymphoma, and a nerve problem called peripheral neuropathy.

The epidemiology study likely to carry the most weight in the TCDD discussion was published in January by Marilyn Fingerhut and her colleagues at the National Institute for Occupational Safety & Health. Her retrospective cohort study of cancer mortality included 5172 chemical plant workers from 12 companies who had worked in areas making products that were contaminated with TCDD. Generally they worked at plants that produced or used 2,4,5-trichlorophenol. Of these workers, 1052 had died, and the study is based on data on their cause of death. Fingerhut also had blood samples taken from 253 workers from two plants for measuring serum TCDD levels. The participants were divided into groups that had less than or more than one year of exposure to TCDD-contaminated materials. A separate analysis was done of individuals who were exposed more than 20 years ago.

John C. Bailar III of McGill University said in an editorial appearing in the same issue of the *New England Journal of Medicine* as Fingerhut's study was published, "Parties on both sides of the continuing debate about the regulation of dioxin exposure will no doubt cite this work in support of their positions." And he was right.

Among the significant conclusions reached by Fingerhut's group is that, for the group that was exposed to TCDD for longer than a year and with more than 20 years' latency, deaths from cancers of all kinds combined were 46% higher than for the general population. "This is an unusual finding for chemical workers," Fingerhut explains. "Chemical worker studies to my knowledge have not shown that. In the few I'm aware of that see excess in all cancers, the excess has been accounted for by a single-site cancer, say lung cancer." Because of the problem of multiple exposures to chemicals among plant workers, Fingerhut explains, TCDD cannot unequivocally be said to be the cause for this observation, but "it appears to us that the most likely explanation is the exposure to chemicals contaminated with TCDD."

"We showed in this paper a striking correlation between serum levels of TCDD and duration of exposure," Fingerhut says. Consequently, it will allow followup studies to assume that workers of longer duration will have higher levels of TCDD in their blood. It is also of note that, with one exception, the Fingerhut study did not find excess cancer deaths from any one type of cancer, including non-Hodgkins lymphoma, which in the past has been associated with TCDD exposure. The Fingerhut report is inconclusive on the issue of TCDD's causing soft tissue sarcoma. Some say her finding of three deaths when less than one was predicted proves the connection. Others say the numbers are just too small to be meaningful. "As time goes on, reanalysis of this study group will be more powerful and it may be that the interpretation will become more clear," Fingerhut says.

To Vernon N. Houk, director of the Center for Environmental Health & Injury Control at the Centers for Disease Control, the issue is mostly decided. "I believe that a conservative interpretation of the Fingerhut study is that if dioxin is a human carcinogen, which I am assuming it is, it is a relatively weak one and is a carcinogen only at extraordinary doses." Houk notes that Fingerhut's study agrees with results from the Ranch Hand

TCDD antiestrogen effect has tumor-fighting potential

After more than 30 years of experience, there is little doubt that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a dangerous chemical to have in the environment. Still, approximately 10 years ago, a few scientists took note of one effect of TCDD in rats and have since been pursuing an interesting and potentially valuable line of research.

As Michael A. Gallo, associate dean for research at the Robert Wood Johnson Medical School in New Jersey, tells it, it all started with a Dow Chemical study by Richard J. Kociba, published about 13 years ago. The study showed an increase in liver cancer in rats exposed to TCDD, "but at the same time there was a remarkable decrease in spontaneous breast tumors and spontaneous uterine tumors," Gallo says. He and others began looking into this effect, wondering if TCDD, which seemed to be reacting with a hormonelike receptor, might be an antiestrogen. If it was, and could somehow be controlled, there was a potential for reducing women's risk of estrogen-dependent breast cancer.

Gallo says early tests in mice confirmed that TCDD did somehow prevent estrogen from doing its job. "One experiment to measure estrogen activity is to inject estrogen into an immature mouse. The uterus will then mature very rapidly. So you end up with an immature animal with a mature uterus. We did it the other way. We injected immature animals with TCDD (an antiestrogen). The untreated mice went on to develop normal uteruses and the animals given TCDD had immature organs." It was clear TCDD was preventing estrogen from acting.

There are two ways this might occur. The first is that the TCDD-receptor complex is actually inducing production of an enzyme that metabolizes estradiol. Gallo says there is some evidence of this, but that it alone is probably not enough to account for TCDD's observed antiestrogenic effects.

Gallo and collaborator Steven H. Safe, of the department of veterinary medicine at Texas A&M University, are working on the assumption that the TCDD receptor is somehow interfering with production of the normal estrogen receptor in cells. "We see changes in the messenger RNA for the estrogen receptors," he says. "It's decreased. And the actual estrogen receptor protein is decreased after TCDD treatment at very low doses." Safe adds that "TCDD also seems to block other stimulatory agents that make breast cancer cells grow."

A common drug used currently as a palliative breast cancer therapy, tamoxifen, has some side effects and, Gallo says, about 15% of the patients put on the drug become susceptible to tumors again after five or six years. "If we had a drug that didn't allow the estrogen receptors to be synthesized in these cancer victims, we wouldn't have to worry about blocking their action," he explains. Thus, if TCDD does functionally block formation of estrogen receptors, there is clinical potential.

Of course, the toxicity of TCDD is such that it would not be used as a drug. However, Safe says they have already synthesized relatively nontoxic TCDD analogs to see if those compounds produce the same responses. "Other dioxins and dibenzofurans that we've made have less toxicity but they still seem to have these potent antiestrogenic effects. Not as potent as TCDD, but still pretty good," Safe says.

study in that persons exposed at moderate levels do not seem to be having excess cancer mortality. He hopes that this, as well as additional research will be used in the future to make better risk assessments. "The federal government has spent more than $400 million to research this compound, in addition to what the companies have spent. If we don't use that new information to modify or support our views on dioxin, then why did we do it?"

The next body of knowledge coming to bear on TCDD's toxicity will be the result of research on animals and at the molecular level. One of the most significant realizations of the past few years is that TCDD cannot be considered by itself. Molecular biology has shown that the action of TCDD in a cell is receptor mediated and that there are a number of dioxinlike compounds that can all have toxic effects.

This receptor-mediated action for TCDD was first discovered in 1976 in Alan Poland's lab at the University of Wisconsin, Madison. Poland and others injected low levels of TCDD into animals and found that one of the first things that happened was the induction of a particular cytochrome P450 microsomal enzyme that oxygenates substrates, such as the carcinogen benzo[*a*]pyrene, that accumulate in fatty tissues. The response depended on the dose and had a great deal of potency. Researchers also noted that other planar chlorinated dioxins, dibenzofurans, and polychlorinated biphenyls (PCBs) could elicit a similar response, although not so strong as TCDD.

The evidence, found first in chicken embryos and tissue culture, then in rats and other animals, looked like classic receptor-mediated transduction, very similar to the way steroid hormones are made in cells. The receptor was recognized as a soluble intracellular protein and was designated the Ah—for aryl hydrocarbon—receptor. One experiment that clinched the presence of this receptor was that mice, inbred to be genetically without the receptor, do not respond with enzyme induction when treated with TCDD. Although it is impossible to say for sure, all evidence points to only one receptor for TCDD, one that begins all TCDD cellular activity.

So TCDD gets into the cell, where it binds strongly to the Ah receptor. It was discovered, however, that this wasn't enough to generate induction of P450. Subsequent research identified another protein, a translocating factor, necessary for the complex to pass from the cytoplasm into the nucleus. This protein has just recently been cloned by Oliver Hankinson and coworkers at the University of California, Los Angeles, department of pathology and the Laboratory of Biomedical & Environmental Sciences at UCLA.

Once inside the nucleus, it seems that several—possibly as many as four or five—of these complexes attach

to a specific sequence on the cell's DNA. This, according to James P. Whitlock Jr. at Stanford University School of Medicine's department of pharmacology, distorts the DNA chain, allowing attachment of other binding proteins. These events lead to the transcription of messenger RNA that forms the cytochrome P450 or other enzymes that might be induced by TCDD. These steps, too, are typical of a steroid induction in cells.

TCDD is now known to affect several genes. The gene for the original cytochrome, which is now called P4501A1, and a second cytochrome, P4501A2, are just two examples. Others include a gene for glucuronyl transferase, glutathion-*S*-transferase, and aldehyde dehydrogenase. Researchers have identified some other genes activated by the TCDD complex but have not fully characterized the mechanisms yet.

Although the enzymes these genes produce are not believed to have anything to do with TCDD toxicity, it is clear that the TCDD complex is the mechanism through which TCDD exhibits a toxic effect. Studies done with the Ah-receptor-defective mice showed that all the different toxicities of TCDD measurable in mice—chloracne (in hairless mice), porphyria, cleft palate formation, teratogenesis, even death—were limited to the mice that had the high-affinity receptor. Structure activity relationship studies also confirm this, according to Whitlock. The more potent the ligand for the receptor, the more potent the biological response. TCDD is the strongest binding of all the dioxins and dioxinlike compounds. The conclusion is that all the biological and toxic effects of TCDD and dioxin-related compounds are mediated through this one receptor.

Exactly what the receptor is, is still unknown. So far, its structure has remained elusive, although some researchers are getting close. Scientists have noted, too, that small differences in the receptor occur among species. They know these receptors have different molecular weights, but they don't know what that might mean with respect to affinity for TCDD. The Ah receptor is similar to the receptor that induces steroid hormones, such as estrogen. Thus, some scientists have put the Ah receptor in the same family as steroid hormone receptors, although others are convinced there are differences.

One scientist working on the receptor model is Ellen K. Silbergeld, adjunct professor of toxicology at the University of Maryland, College Park. "TCDD's interaction with this receptor is very powerful," Silbergeld says. "From my perspective, it must be doing something very important in normal cell physiology. I take it as an assumption that somewhere there is a natural compound that responds to the Ah receptor and transduces a set of events very important in normal cells." Silbergeld and other researchers point out that toxicology research has often found the unnatural ligand for receptors before it finds the natural compound. Some scientists believe that finding this endogenous ligand, which TCDD apparently mimics, for the Ah receptor will be a central issue for TCDD research. The receptor was found for opiates before the body's own endorphins were discovered, for example.

The very high specificity that TCDD and other dioxin-like compounds have for the Ah receptor is key to the development of what is called toxicity equivalents, E cause other dioxins, dibenzofurans, PCBs, even logenated naphthalenes bind to the receptor ar cytochrome P4501A1, it is assumed that they can cau the same range of toxic effects as TCDD. Because TCD has the strongest affinity, it is given a toxicity equivale cy factor of 1, and all others are being compared to EPA has adopted a list of factors for calculating toxicit of these mixtures of compounds. For instance, a pe tachlorodibenzo-*p*-dioxin with four of its chlorines the 2,3,7, and 8 positions is given a value of 0.5. C tachlorodibenzo-*p*-dioxins have a toxic equivalency zero.

The implications for measuring health effects of a these chemicals in humans and animals is significant. means that no longer can TCDD really be considere alone, but only as part of a potential problem. Accor ing to Linda S. Birnbaum, director of the environme tal toxicology division of the EPA Health Effects R search Laboratory in Research Triangle Park, N.C "There are suggestions that coplanar PCBs may be, fact, responsible for much of the toxicity equivalency human serum. In other words, in industrialized cou tries, the background level of TCDD in people may b 7 ppt, and if you add the toxic equivalencies of all th other dioxins and furans, it gets up to about 30 ppt. you now add the toxicity equivalents of all the PCB you might say that people are actually walking arour with 100 ppt of dioxin equivalents in their body."

While arguments will persist on what toxi fec these levels of contaminants have on people, t a already significant data on their effects on fish an wildlife. Philip M. Cook of the EPA Environmental R search Laboratory in Duluth, Minn., reports that trou in Lake Ontario have an average of 35 ppt TCDD. Rich ard E. Peterson at the Environmental Toxicology Cente and School of Pharmacy at the University of Wisconsi Madison, reports that concentration of 65 ppt TCDD eggs of lake trout can cause 50% mortality. Peters says newly hatched lake trout exposed to TCDD in t egg stage are the most sensitive to the lethal effects TCDD compared to any mammal, bird, or fish spec ever investigated. Cook says, though, that TCDD co centrations were higher in the past. "The tendency h been for the concentration [in fish] to come down," h says. There are no archived fish samples for compar son, but Cook guesses peak concentrations occurre sometime in the 1960s. This observation agrees wit the finding by Kang that TCDD levels in humans ma be dropping too.

One of the problems facing EPA is how to measur the buildup of TCDD in organisms as the contaminar moves up the food chain. The agency presently a sumes a bioaccumulation factor of 5000 for TCDD lev els in water. This may be too low, argues Peter deFur, scientist working for the activist organization Enviror mental Defense Fund, who says EPA is using old dat EDF recommends that the factor be raised to ea 50,000. On the other hand, Cook says their b est mate for bioaccumulation of TCDD in Lake Ontar trout is on the order of 160,000.

Though death is obviously the worst biological effe

of TCDD and dioxinlike compounds, other problems are serious as well. Cook says the levels of TCDD in Lake Ontario are probably leading to a lack of natural reproduction in fish. At the U.S. Fish & Wildlife Service in East Lansing, Mich., Timothy Kubiak is investigating the reproductive effects of TCDD on mink, the species most sensitive to the compound after guinea pigs. He says by examining the toxicity of each of the individual congeners in mink, and, depending on several factors, TCDD may contribute to only about 5% of the total toxicity, most of which comes from PCBs. Mink are a good species to study, Kubiak adds, because their sensitivity makes them a good sentinel for changing concentrations of pollutants.

Silbergeld: physiology of Ah receptor

One toxic effect may be sexual aberrations in birds and animals from exposure to dioxinlike compounds. Theo Colborn of the World Wildlife Fund in Washington, D.C., is monitoring research on the effects of organochlorine compounds on wildlife, and she says that researchers are reporting instances of hermaphroditic offspring, such as male birds with oviducts, and abnormal female-female pairings of birds. Colborn contends that this is happening because TCDD and the dioxinlike compounds are messing up the estrogen receptors on developing embryos at critical times. "One dose of TCDD to rats on day 15 of pregnancy, that's about when sexual differentiation occurs, found a dose response in demasculinization and feminization of male offspring," she says.

All the information on animal toxicity and the data on molecular chemistry are going to be taken into account during a major effort, now under way, that EPA is making to revise its programs for regulating TCDD. After publication of the Fingerhut study, and the scientific agreements that came out of the Banbury conference, EPA's Reilly told the agency it was time for a reassessment.

William Farland, head of EPA's Office of Health & Environmental Assessment for the Office of Research & Development, says, "The review includes five major activities. First is the evaluation of the biologically based dose response model for TCDD, taking into account the idea that TCDD is known to bind to a specific receptor in cells. Second is an evaluation of the science in various aspects of health effects, including carcinogenicity, reproductive effects, immunotoxicology, acute and chronic effects, and human epidemiological data. The final three items are a health research component, an ecological research component, and an exposure reassessment component." The entire project is supposed to be completed by May 1992.

Reconsideration of its cancer model represents a major break with tradition for EPA. The present linearized multistage model for carcinogenicity does not work for receptor-mediated molecules because it does not allow for a threshold below which cancer would not occur. According to Farland, the EPA guidelines, however, do permit the agency to change its model. "The 1986 cancer guidelines suggest that one ought to choose a dose response model with a biological rationale. TCDD provides a good example of a chemical [for which] a lot of good studies have been done, and we ought to be able to bring this information into our dose response analysis. So we think this is consistent with the agency's advice all along and is one of the best opportunities we have to put that advice into practice."

EPA's Birnbaum is heading the agency's research on health effects. "Basically there are three areas we are trying to address here. First, we are trying to define the dose response curves, focusing on the most sensitive toxicological endpoints. Next, we are looking at enzyme induction, specifically cytochromes P4501A1 and P4501A2. Finally, we are trying to find out where people are with respect to these responses."

Birnbaum: sensitive indicators needed

TCDD has many molecular effects that can now be measured. Ligand binding to the Ah receptor, nuclear occupancy of the activated complex, cytochrome induction, and immunotoxicity are examples. But each of these seems to happen at a different level of TCDD exposure. Birnbaum says EPA is focusing on the low-dose region of the response curves for these markers, looking for the most sensitive endpoints. When it comes to measuring effects of TCDD, cancer may be a poor indicator. Birnbaum says it looks like im-

munotoxicity may be the most sensitive indicator of TCDD effects, and this is clearly a problem.

Cytochrome induction has been considered one of the easiest responses to detect. The P4501A1 enzyme can be induced in most cells and animals. Measured in terms of the action of aryl hydrocarbon hydroxylase, the cytochromes are important because they can actually metabolize some environmental chemicals into suspected carcinogens, Birnbaum says. "These cytochromes are present in high concentration in the liver in response to TCDD and related chemicals," she says. It is interesting that the liver was where Kociba at Dow recognized TCDD as a carcinogen.

A simple test for the presence of cytochrome P4501A2 is being worked on. According to Birnbaum, the hydroxylase acts at a specific site on the caffeine molecule. By labeling caffeine with carbon-13 at that site and administering it to people, the 1A2 cytochrome can be detected because ^{13}C-labeled carbon dioxide will be exhaled. "We are doing this now in experimental animals," Birnbaum says.

EPA's lab will be assessing the endpoints for 11 different responses in the same animal. The idea is to see at what dose responses occur. Birnbaum says the data will be used to develop a risk model that might tell EPA just where humans fit into the overall scheme of toxicity of TCDD and dioxinlike compounds. If one assumes that people are walking around with about 100 ppt of TCDD toxicity equivalents, the assessment would be a guideline as to what response might come from that. "We may see that 100 ppt is orders of magnitude below the toxicological inflection point," Birnbaum says, "and therefore not of great concern. On the other hand, if the current exposure levels put us right near that inflection point, then any additional exposure would be undesirable." She adds that EPA particularly needs to look at the issue of sensitive populations, such as subsistence fishermen and nursing infants, that might receive doses 10 to 20 times higher than the overall population.

A great deal has been made about the issue of there being so great a species difference in response to TCDD, Birnbaum says. However, for most toxicological endpoints, such as death, researchers can find a species outlayer. In this case it may be guinea pigs, which die at exposures of less than 1 μg per kg body weight. For the related hamster, the dose at which half die may reach as high as 5000 μg per kg body weight. "But most species cluster their sensitivity somewhere within a 10-fold range," she says. "If you take other endpoints for TCDD, say developmental toxicity, the dose that will kill the developing fetus is essentially within an order of magnitude in the guinea pig, hamster, rat, and mouse. If you look at enzyme induction, the dose that causes a response in these animals is essentially the same. So lethality has been sort of a red herring."

Looking at the endpoint information on humans shows that they fall into the same range of sensitivities, Birnbaum says. "For enzyme induction and chloracne, humans respond similarly to experimental animals. In in-vitro experiments, the concentrations of TCDD that result in cleft palate formation in the rat and in the human are essentially the same. For cancer, the recent study by Fingerhut is at least compatible with the hypothesis that the blood levels in the group that had increased cancer were similar to the blood levels in the rats that developed cancer in the Kociba study."

Birnbaum thinks there is no reason to believe that people are different from animals in their response to TCDD. "I think the Seveso data tell us that, in terms of lethality, we are not guinea pigs. At the highest doses received by residents near Seveso, if they had been guinea pigs, there would have been some deaths. But we never reached the levels of TCDD that would have killed rats or mice or monkeys or dogs or rabbits or minks or anything else."

EPA's review will bring up to date the scientific reasoning for TCDD toxicity. By merging the human epidemiology data, the animal toxicity information, and the molecular biology, a better level of understanding and maybe a firmer basis for regulation of this contentious chemical will emerge.

Silbergeld and Birnbaum both think the regulatory number for TCDD may not change much. Even though the response level for cancer is high, the response level for immunotoxicity may be very low, and the current safe intake calculation of 0.006 picogram per kg body weight per day may be in the right range. CDC's Houk has commented that the intake number seems too low. Given the history of TCDD, it is likely that politics and emotion will have as much say in the end as does science.

Politics and emotion have a lot to do with the public's fear of dioxin. Those embroiled in the public controversy have diverse views on why TCDD remains so persistently in the forefront of people's concerns. Stanford's Whitlock believes it is because the compound has become synonymous with horrible scenes of birth defects and cancer. "It has become a sort of prototype for certain groups who are concerned about the environment," he says.

Several people place the focus on the Vietnam veterans. The Air Force's Wolfe, for one, thinks, "It was so closely related to the Vietnam experience and the ill treatment a lot of the returning folks got." But he also says, "It is sort of a flagship issue of all environmental problems."

Houk says attention is rapt because of those who insist that TCDD be labeled the most toxic substance known to man. Joseph Walker from the Chlorine Institute concurs on that point, adding that it also may be because "dioxin" looks and sounds like "toxin."

But uncertainty may be the biggest reason people's concerns haven't been eased by science. NIOSH's Fingerhut speculates that people have never felt reassured by the information coming out. "It is hard to get answers for a problem like this, and they are long in coming," she says. Banbury conference organizer Gallo agrees. "I think the scare comes from some people saying there is no problem and others saying this is the most heinous compound man has ever created." At Maryland, Silbergeld blames the uncertainty on the government. "Primarily it's the government's fault that they can't come to a decision on TCDD regulation and stick to it." □